

Attorney Docket No. 3817.14-1  
Customer No. 23308

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listing, of claims in the application:

**Listing of Claims:**

1. (Currently Amended): A protein binding assay for measuring inositol 1,4,5-triphosphate (IP<sub>3</sub>) in a sample employing as reagents a conjugate of IP<sub>3</sub>, a detectable label joined through a bond or linker at the 2-hydroxyl position of said IP<sub>3</sub>, and as a binding protein a ~~truncated~~ 226 – 578 amino acid extracellular portion of ~~an mouse~~ inositol 1,4,5-triphosphate receptor (IP<sub>3</sub>R) having at least about 200 times the affinity for IP<sub>3</sub> than the intact IP<sub>3</sub>R, wherein said conjugate and IP<sub>3</sub> in the sample compete for binding to said binding protein and the amount of bound or unbound conjugate will be related to the number of binding proteins bound by IP<sub>3</sub> in said sample, said method comprising:  
  
combining in an assay medium said sample, said conjugate and said binding protein and incubating said mixture for sufficient time for complex formation of IP<sub>3</sub> and said conjugate with said binding protein; and  
  
detecting the bound or unbound label as a measure of the IP<sub>3</sub> present in the sample.
2. (Original): A protein binding assay according to Claim 1, wherein said assay is in a homogeneous format.
3. (Original): A protein binding assay according to Claim 1, wherein said sample is a cellular lysate, and wherein said cellular lysate has been treated to block kinases and phosphatases and prepare said sample for said assay.

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4. (Currently amended): A protein binding assay according to Claim 1, wherein said binding protein is of not more than about 600 amino acids and comprises at least amino acids 226 – 578 of the mouse IP<sub>3</sub>R Type 1 fused to glutathione-S-transferase.
5. (Canceled).
6. (Original): A protein binding assay according to Claim 1, wherein said binding protein is a fusion protein of up to about 1.5kD amino acids.
7. (Original): A protein binding assay according to Claim 1, wherein said label is a fluorescer.
8. (Original): A method according to Claim 1, wherein the order of addition of reagents is: (a) combining said sample with said binding protein; and (b) adding said conjugate, with incubating after (a) and (b).
9. (Withdrawn): A protein binding assay for measuring IP<sub>3</sub> in a sample using a homogeneous format, employing as reagents a conjugate of IP<sub>3</sub> and an ED of from 37 to 60 amino acids derived from  $\beta$ -galactosidase joined through a linker at the 2-hydroxyl position, and a truncated extracellular portion of an IP<sub>3</sub>R having at least about 200 times the affinity for IP<sub>3</sub> than the intact IP<sub>3</sub>R, said method comprising:  
  
combining in an assay medium assay components in the following order:  
said sample, said binding protein, said conjugate and EA, and incubating  
after each combining for sufficient time for complex formation between said  
assay components;  
  
adding substrate for said  $\beta$ -galactosidase; and  
  
detecting the turnover of said  $\beta$ -galactosidase of said substrate as a measure  
of the IP<sub>3</sub> present in the sample.

10. (Withdrawn): A protein binding assay for measuring  $IP_3$  in a sample using a homogeneous format, employing as reagents a conjugate of  $IP_3$  and a fluorescer joined through a linker at the 2-hydroxyl position, and a truncated extracellular portion of an  $IP_3R$  having at least about 200 times the affinity for  $IP_3$  than the intact  $IP_3R$ , said method comprising:

combining in an assay medium assay components: said sample, said binding protein, and said conjugate, and incubating for sufficient time for complex formation between said assay components; and

detecting the change in fluorescence polarization as a measure of the  $IP_3$  present in the sample.

11. (Withdrawn): A method according to Claim 10, wherein said linker is an aliphatic group of from 4 to 20 carbon atoms.

12. (Withdrawn): A method according to Claim 10, wherein said fluorescer emits at a wavelength greater than about 500 nm.

13. (Withdrawn): A method according to Claim 10, wherein said fluorescer has a polarizability of less than about 60mP.

14. (Withdrawn): A protein binding assay for measuring  $IP_3$  in a sample employing as reagents a conjugate of  $IP_3$  and a detectable label joined through a bond or linker at the 2-hydroxyl position, and a truncated extracellular portion of an  $IP_3R$  having at least about 200 times the affinity for  $IP_3$  than the intact  $IP_3R$ , said method comprising:

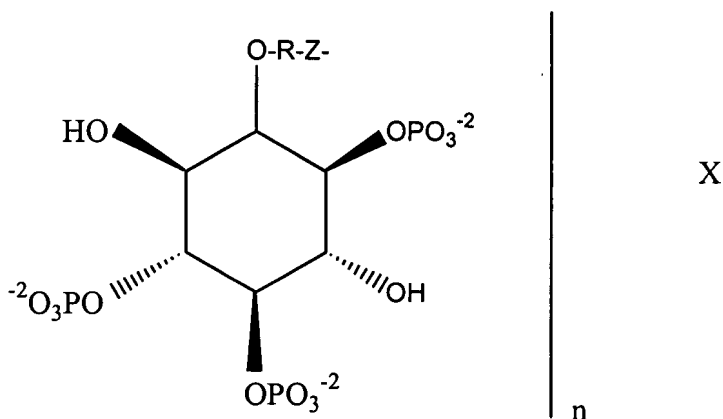
combining in an assay medium said sample, said conjugate, said binding protein and a chemical reductant and incubating said mixture for sufficient time for any  $IP_3$  and said conjugate to bind to said binding protein; and

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detecting the bound or unbound label as a measure of the  $IP_3$  present in the sample.

15. (Withdrawn): A protein binding assay according to Claim 14, wherein said chemical reductant is a thiol.

16. (Withdrawn): A compound of the formula:



wherein:

R is a neutral linking group of from 4 to 20 carbon atoms bonded to the oxygen through a saturated carbon atom or carbonyl;

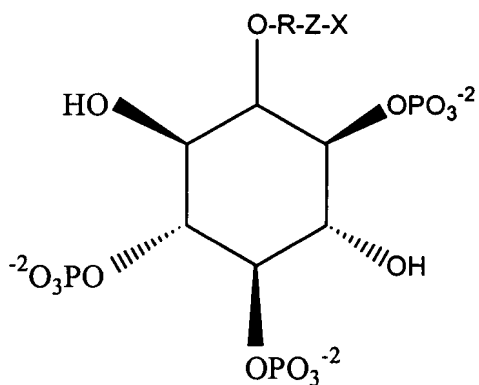
Z is a functionality for linking X to the oxygen at the 2-position;

X is an enzyme donor fragment of  $\beta$ -galactosidase of from 27 to 60 amino acids; and

n is 1 or 2.

17. (Withdrawn): A compound of the formula:

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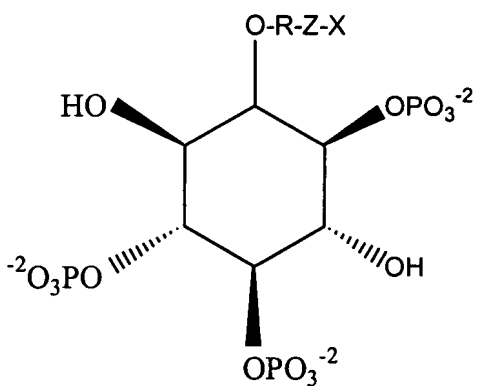
wherein:

R is a neutral linking group of from 2 to 20 carbon atoms bonded to the oxygen through a saturated carbon atom;

Z is a functionality for linking X to the oxygen at the 2-position; and

X is a fluorescer.

18. (Currently amended): A kit comprising a compound



wherein:

R is a neutral linking group of from 2 to 20 carbon atoms bonded to the oxygen through a saturated carbon atom;

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Z is a functionality for linking X to the oxygen at the 2-position; and

X is a fluorescer, enzyme acceptor for said enzyme donor and a ~~truncated~~  
226 – 578 amino acid extracellular portion of an mouse IP<sub>3</sub>R having at least  
about 200 times the affinity for IP<sub>3</sub> than the intact IP<sub>3</sub>R.

19. (Canceled).

20. (Withdrawn): A kit for performing an IP<sub>3</sub> assay comprising a conjugate of  
IP<sub>3</sub> and a detectable label joined through a bond or linker at the 2-hydroxyl  
position, a truncated extracellular portion of an IP<sub>3</sub>R having at least about  
200 times the affinity for IP<sub>3</sub> than the intact IP<sub>3</sub>R and instructions for  
performing said assay.

21. (Withdrawn): A kit according to Claim 20, further comprising a thiol  
reductant.